Indian J Physiol Pharmacol 1998; 42 (2) : 189–204

REVIEW ARTICLE

USE OF CHICK EMBRYO IN SCREENING FOR TERATOGENICITY

ANITA KOTWANI

Department of Pharmacology,
Maulana Azad Medical College,
New Delhi – 110 002

(Received on July 7, 1997)

Abstract: A teratology screening system would detect agents hazardous to the conceptus before they can perturb embryonic development in humans. The back log of untested chemicals and the rate at which new substances enter the market exceed the developmental effects testing by standard *in vivo* method. Thus, cheaper, quicker *in vitro* systems afford a unique opportunity for investigating the direct interaction of substances with developing morphogenetic system (MGSs), since maternal influences are excluded. As a carrier of a complete set of MGSs, the chick embryo in ovo manifests an advantage over those *in vitro* systems that employ isolated embryos or embryonic tissues that have only limited survival. Under controlled experimental conditions including standardization of subjects, administration technique and mode of evaluation, according to the basic principles of teratology, the chick embryo test is demonstrated to be reliable and to afford quantifiable end points for evaluation. Individual compounds, mixtures of compounds and agoinst and antagonist can easily be administered and tested. The chick embryo possesses its own basic enzyme-catalyzed drug - transformation capacity and moreover, it can be used for screening specific human metabolites. Different newer techniques e.g. chick embryotoxicity screening test (CHEST), Chick embryo blastoderm model etc are described in detail. Chick embryo fulfills all the criteria which a test should have at a lower level of tier system in teratological studies i.e. modest laboratory equipment, moderate skill, minimal expenditure of time and money, ease of accessibility of embryo, known embryological development, possibility of experimenting on a large scale for statistically valid results and whole animals are also not required.

Key words: chick embryo teratogenicity screening

A teratology screening system would detect agents hazardous to the conceptus before they can perturb embryonic development in humans. The currently accepted tests for teratoginicity comprise the administration of the test agent to pregnant rodents or lagomorphs with examination of the progeny near term. These whole animal *in vivo* tests have developed to a relatively standardized form and are utilized world wide (1). They suffer, however, from two serious problems : they are expensive and

time consuming to perform, and the extrapolation of the results to the human population is confounded by the well known species variation in teratogenic response (2).

Need for other teratogenicity tests

The number of compounds which must be tested for teratogenicity has increased dramatically with the continuous development of therapeutic, cosmetic and food additive chemicals. It is clearly unrealistic to attempt to perform complete *in vivo* teratogenicity tests on each and every one of these chemicals. Thus cheaper, quicker, more efficient, but nevertheless reliable tests must be developed. These tests may sometimes function only as a pre-screen for the detection of compounds which may require further, more exhaustive, testing. In other cases, a risk benefit decision may be made based upon the result of *in vitro* testing (3). Many *in vitro* techniques have been evolved but till date no method has been able to replace whole animal testing, as the regulations in many cases require specific tests and the fear of litigation is also there if testing is not done by standard methodology. Moreover the concept of development of *in vitro* methods is very new and they await validation and standardization before they gain general acceptance (4). The international group of experts have reached a consensus regarding the desirability of developing new and flexible guidelines. These include the possibility of considerable reduction in duration and size of studies and number of animals used where there is an indication that a low hazard potential exists (5).

The test systems

In the last few years, a number of other systems have been proposed as possible screening tests for teratogenicity. The available *in vitro* systems are mammalian organ culture (6); vertebrate embryos e.g. chick (7), fish (8), and amphibian embryos (9); invertebrate system like drosophila (10), cricket (11), hydra (12); organ culture (13) and cell culture system (14).

As is apparent, a number of possible teratogen screening systems can be used for the rapid identification of potential teratogens. At present, no single *in vitro* system is likely to supersede animal testing, but one can foresee the development of a successful battery of tests for identifying compounds for further *in vivo* testing. Logistically, it will never be possible to test chemicals in wide spread use if we have to rely solely on the mammalian bioassay, which uses small treatment population and takes six to ten months to complete.

Chick embryo in screening for teratogenicity

Basic advantages of chick embryo : Chick embryos in a proper state of preservation and of the stages desired can readily be secured and prepared for study. Used as the only laboratory material in a brief course, they afford a basis for understanding the early differentiation of the organ systems and the fundamental processes of body formation common to all groups of vertebrates. The developing chick embryo is one of the most extensively used living system for biological research. The availability of fertile eggs, the rapid growth of the embryo and the ease in manipulating

it, have made the chick embryo a model system for morphological, biochemical and functional studies on growth, differentiation and organogenesis. During the 21 day period when the egg progresses from a single cell to a hatched and self sufficient individual are concentrated most of the complex problems of development and differentiation (15).

Chick embryo has contributed enormously to experimental embryology and there is a vast amount of literature describing the development and their use as model system (16, 17). Structures of the egg at the time of laying is shown in Fig. 1 and the various stages of development (18) as suggested by Hamburger and Hamilton (1954) are still used by scientists for development of chick embryo corresponding to different hours of incubation. Teratology is defined as a science dealing with the causes, mechanisms and manifestations of developmental deviations of either structural or functional nature. So any change in the normal development (growth retardation, defect or death) is considered as teratogenic effect.

The chick embryo is commonly used in pharmacological and toxicological research owing to its ready availability and ease of handling and because many of its responses have predictive value for other species (19).

Lacunae

Despite its extensive use in teratological research (20), the chick has never been

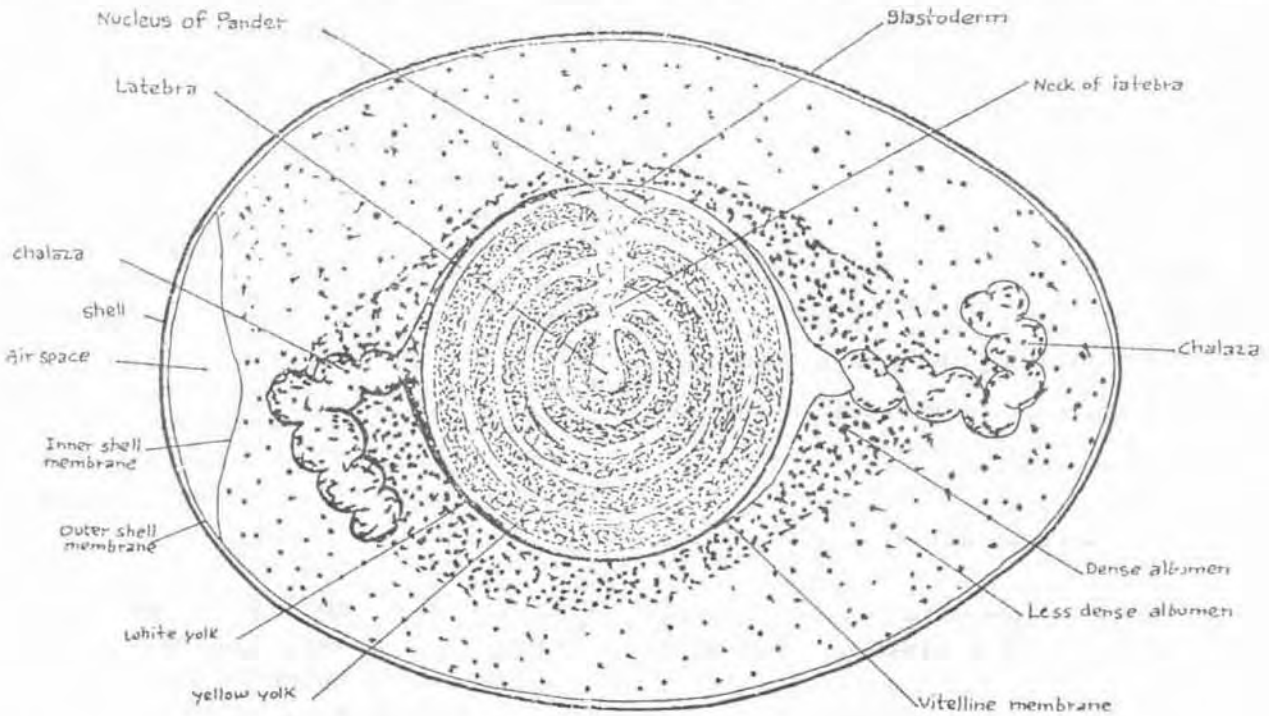

Fig. 1 : Schematic diagram of the hen's egg in longitudinal section showing the relations of the various parts of the egg at the time of laying.

employed in routine procedures (21). When the first official guidelines were established in 1966 both theoretical and technical knowledge regarding the chick embryo were in a preliminary state. Yolk sac injections were used and results were evaluated after hatching (22–24). Thus, re-introduction of the earlier air-sac administration technique (25–26) could not avert widespread unfavourable evaluation of the avian model, for example in 1967 the World Health Organization stated that "The Chick embryo ... for screening of drugs for teratogenicity .... is not recommended" (27). The main objections to the use of the chick embryo in teratological testing were as follows.

1. Absence of the appropriate (i.e. mammalian) maternal fetal relations.
2. Pharmacokinetic dissimilarities inherent in the closed character of the avian egg with respect to injected xenobiotics.
3. High non-specific sensitivity resulting in an unjustifiable number of false positive results.

On the basis of extensive experimental evidence (28), it may be argued that these three objections have arisen mainly from

a) neglecting the principles of teratology
b) poor standardization of test subjects and
c) inadequate administration technique

The avian ovum as an *in vitro* system in teratology

Chemical embryotoxicity is a function of concentration of a substance and/or its metabolities in a target developing (morphogenetics) system (MGS). In reality, however, the situation is much more complicated, the manifestations of embryotoxic potential depends on the dose, critical period and sensitivity of the MGS at the time of administration and also upon a metabolic system that transforms a substance to either active or inactive metabolites. In viviparous animals, the maternal drug metabolizing system represent the major source of the mammalian intraspecies and interspecies variation in response to teratogens (29). The role of placenta, in this respect, was demonstrated as having been over estimated, for most foreign compounds enter the fetus by passive diffusion (30).

Devoid of maternal metabolic influences, the *in vitro* systems afford a unique opportunity for investigating the direct interaction of a substance with developing MGS. For extrapolation purposes, however, the specific metabolism of substances is frequently needed and many *in vitro* systems require direct testing of isolated metabolites or the addition of isolated metaboling enzyme systems to address this issue. The avian embryo, however, possess *in vivo* a drug metabolizing capacity, at least from day 2 of incubation onward. This was clearly demonstrated for cyclophosphamide (31) as well as for other substances (32). Besides having its own enzyme catalyzed transformation capacity, the avian embryo system can easily be used for the detection of stable metabolites occurring in human serum (33).

Serious problems, however may arise in delivering a test compound in a standard concentration to embryonic tissues. It has been repeatedly demonstrated that when using the ordinary yolk sac injections one cannot be sure of when and in what form

and concentration, a substance reaches the embryo (34, 35). Thus when this administration technique is used, the target MGS are exposed over different critical periods with unknown doses. The reliability of such an experiment must undoubtedly be low, unless very large numbers of specimens are used (36). However, when using intraamniotic application of small amounts of substances dissolved in volume up to 10 µl the concentration in embryonic tissues develops a pattern similar to the mammalian embryo after po or iv administration (32) and a dose response relationship for increasing occurrence of abnormalities is seen over the entire dose range.

Another potential disadvantage of the avian embryo assay is the standardization of experimental subjects. There is a great variability of developmental stages in various groups of embryos of the same stock. Thus, when an administration technique does not allow the experimenter to select embryos for proper development and staging the reproducibility of an experiment cannot be expected. For this reason more accurate techniques of compound administration and developmental staging must be followed if the chick embryo method is to be validated for embryotoxicity testing.

The avian embryo : a carrier of morphogenetic systems

Malformation rarely involve the whole embryo, since as a rule a characteristic pattern develops that is dependent on the developmental stage at administration, the species used, and the nature of the substance and dose level. Based on this research experience a theory was outlined that presents the embryo as a developing mosaic of morphogenetic systems (37). Responding to a large extent independently the MGSs can even be used separately (e.g. organ cultures), when detecting the embryotoxic potential of substances. In this way, an aberrant development may be recorded at the most desirable time period, e.g. just before the onset of degradation or compensatory process or before transformation to another type of effect. The investigation must not be limited, however to a single MGS because the sensitivity of a particular cell population to a test substance may be altered during the incubation period (e.g. by differentiation of specific cell receptors). As a carrier of a complete set of MGSs, the chick embryo manifests a remarkable advantage over those *in vitro* systems that employ isolated embryonic tissue with limited survival, as well as those systems lacking a developing vascular bed, a frequent target for teratogens (38).

Technical aspects in relation to teratology in chick embryo

Newer techniques have been evolved for standardizing the drug delivery to chick embryo, hence chick embryo is coming back as a screening method for teratogenicity. Moreover as the list of chemicals which must be tested for potential of teratogenicity has grown to an intolerable burden, the chick embryo has received more favourable review. Several scientists have described protocols in which the chick is utilized in a predictive test for teratogenicity. Following is a brief survey of the various technical aspects of experimentation related to teratology in the chick embryo.

Culture of whole embryos *in vitro*

The introduction of more and more pharmaceutical compounds into therapeutics require in depth knowledge of their properties. Disasters like those caused by thalidomide emphasized the necessity of testing new substances in order to vouch for their safety. It is of course impossible to test a pharmaceutical preparation for its teratogenic activity in man. On the other hand, it has been demonstrated that animals are more sensitive than human beings.

To screen a substance for its type of toxicity, the growing chick embryo *in vitro* seems an extremely useful tool. It has a convenient size and its organs are easily observable during culture, so that any modifications in organ or tissue relation can be followed. Many embryologists proved that culture methods are advisable for explaining early morphogenetic events and there is no doubt that pharmaceutical and chemical products could be efficiently tested in that manner (39).

Waddington (1932) introduced the *in vitro* culture of chick embryo in a watch glass containing a plasma clot with embryo extract (40). Spratt (1947) replaced the plasma clot by a saline agar albumin medium (41), while New (1995) cultivated embryos on albumin (42).

Specific teratogens supposedly active at an early stages of development can also be tested by culture technique. Nevertheless, chick embryo culture presents a disadvantage. According to the technique perfected by Wolf and Simon (1955), the survival of explanted embryos is limited to 4 days (43). Moreover, their growth is often disturbed through the tensile strength of the semisolid medium, so that distortions of the embryonic body occur and may alter the interpretations of the results.

An intermediate culture technique was introduced successfully by Dunn (44). They perfected an *in vitro* shell less culture of chick embryos from days 3 to 21. The mean morphological stage reached by culture embryos was 15 days. But other disadvantages became obvious such as incomplete enclosure of the egg content by the chorioallantois, preventing the absorption of albumin into the amnion, or growth retardation from the lack of shell calcium. No teratogenic tests were attempted by these authors but it would certainly be possible to study teratogens by this means and for a longer period than by using the classic *in vitro* culture medium.

Few modified *in vitro* culture techniques have been developed and various researchers have studied the effect of teratogen on early development of chick embryos. Kucera and Burnand (45) have done work with *in vitro* culture of avian embryos and designed an artificial egg, i.e. a transparent chamber in which in the presence of an adequate medium, the development of the embryo can be continuously observed for 4 days. They have defined the relevant qualitative and quantitative criteria of normal development and tested this culture system by using six relatively well known chemicals. On the basis of these experiments they have proposed a simple, rapid and economical method for routine screening of chemoteratogens. They have also studied

the effects of dexamethasone and diphenylhydantoin (46). In brief the procedure followed is - The chick embryos are pre-incubated for 20 hours (h) at 37.5°C and 60% humidity, the corresponding development stage is stage 5 (HH). The development of the embryo takes place in the central transparent area pellucida. The latter is surrounded by the area opaca, heavily loaded with yolk particles. The two areas together form the discoidal blastoderm, the periphery of which is attached to the vitelline membrane. The chick embryo at 20 h corresponds to a human embryo about two weeks old.

A large portion of vitelline membrane with the attached blastoderm is excised from the yolk and transferred to a transparent silicone chamber. The preparation is turned upside down and spread over the ring protruding from the bottom of the chamber. The chamber is closed by a perspex lid and incubated at 37.5°C. Development is observed under a binocular microscope. Drugs are dissolved in the culture medium.

The growth and morphogenesis of the embryos are evaluated after 42 h and compared to the morphological criteria and dimensions characterizing the stage 15 (HH). In uncertain cases, additional evaluations are made after 66 and 90 h. The quantitative and qualitative parameters are introduced into a VAX computer and analyzed using the 'oracle' data exploitation system. The evaluation of one drug is complete in 3 weeks. They have tested 8 drugs and shown that chick embryo seems to react to drugs similarly to other vertebrates. The concentrations to which it responds are comparable to those found in other *in vitro* or *in vivo* systems and when these concentrations are reached in man, they may actually induce toxic effects or malformations (methotrexate, DPH, phenobarbital and dexamethasone). Furthermore, malformations produced by a given drug in the chick are similar to those described in other species including man. Hence, this test is simple, economical and suitable for a rapid preliminary screening. Even though it cannot eliminate the experiments on mammals, it can considerably reduce the number of animals used.

The chorio-allantoic membrane (CAM) of chick embryos is a suitable model for the study of the vascular response to implanted tissue grafts (47) or tissue extracts (48) and to various drugs (49). There are two different techniques by which CAM can be exposed for such purposes - either a piece of the egg shell adjacent to the embryo is removed (windowing) or the embryo with the yolk sac and albumin is explanted into cultivation vial.

Auerbach et al (1974) described a simple procedure for long term cultivation of chick embryos explanted into petridishes (50). This technique permits rapid and ready observation of large number of fertilized eggs. However, a substantial proportion of the cultivated embryos in petridishes are lost within the first three days apparently as a result of yolk membrane rupture.

To reduce the yolk membrane tension, Dunn et al (1981) have used a plastic bag suspended in a tripod. This method

improves the short term survival considerably (i.e. during the first three days) following explantation, but the cultivation vials are very space consuming (51).

In an attempt to maintain a high survival rate and at the same time to be able to use a large number of embryos in each experiment, Jakobson et al (1989) have used 200 ml disposable plastic coffee cups with rounded bottoms as cultivation vials. The cups are used intact or cut to about half the original height but are otherwise unprocessed, plastic petridish tops or bottoms are used to cover the embryos. They are incubated in stacks of two or three. This method is referred to as the "cup-egg" method (52). In these experiments, eggs are usually kept at room temperature for one day and then incubated at 37°C for three days before the explantation. They are neither washed nor disinfected. The egg shells are cracked with a knife and the contents are carefully placed in the cultivation vials. All explanted embryos are incubated for three days at 37°C in an incubator with saturated humidity and 3% CO_2. The chick embryo CAM is a convenient model for the study of vascular development. Explanted embryos can be cultivated on a large scale at a reasonable cost. The model may therefore be useful as a screening method for the study of vascular responses to various compounds and implanted tissues.

Chick embryo (in ovo) for screening of embryotoxicity

Several authors have described protocols in which the chick is utilized in a predictive test for teratogenicity. Those of Karnofsky (53), McLaughlin et al (23), Gebhardt (7) and Wilson (21) are essentially similar. White Leghorn eggs incubated in commercial apparatus at 30°C are usually used. To administer the test agent, a hole is bored in the egg which may be subsequently released with wax or paraffin. The test agent may be administered to the yolk sac, subgerminal cavity, allantois, amnion or air chamber depending upon the physicochemical properties of the compound and the individual preference of the investigator. Opinions on the most appropriate treatment time vary from 0 hours of incubation (23) to 30 hours (21), 48 hours (54) or 96 hours (7). The chick may be examined for abnormalities at any time during incubation at hatching or may be allowed to mature to evaluate functional normality. There are many reports for presumptive teratogens and their antagonists can be administered at specific stages of development and the subsequent morphological, physiological and biochemical responses monitored (55–59). Several avenues of administering test agents have been used: immersing the egg totally in the test solution (57), injecting on to the air chamber (55) and injecting into the yolk. The latter route has been most widely used. However, very young embryos (less than 4 days) are reportedly less susceptible to agent delivered in this way than older embryos (62–63).

The first 72 hours of chick embryogenesis are fundamental to the success of subsequent development. It is during this period that all the organ systems are laid out in rudimentary fashion and in proper relationship to one another (64). Thus the most profound effects of teratogens should be exerted on embryos of 72 hours and younger.

Although older chick embryos (i.e. at 2 or more days of incubation) have been used effectively in many studies, the use of younger embryo as model system is limited severely by the fact that windowing alone during the first day of incubation is highly teratogenic, resulting in predominately dysraphic (open) defects of the central nervous system (65–67). Thus it is impossible to obtain reliable information on the specific effects of suspected teratogens on the early development of the central nervous system using the standard procedures of windowing. Fisher and Schoenwolf (1983) have described improvement in the standard methodology of windowing and treating eggs containing early chick embryos (68). They have incubated the eggs for 24 hours and then windowed, by first piercing the blunt end of each egg with a needle and then cutting a small window (1 × 0.75 cm) directly above the embryo with a dental drill. Eggs containing apparently normal blastoderms are used for further treatment. The air space introduced over the embryo by windowing is filled with albumin or 0.9% saline (0.5–1 ml albumin or saline was added to raise the blastoderm and flush it against the inner surfaces of the shells). Windows are then sealed with tape and eggs are rotated by 180° and reincubated for an additional 24 hours. By this technique, the authors showed that the defects of the neural tube are virtually elliminated if the airspace introduced over the embryo is filled with albumin or saline.

Based upon the principles and theory of morphogenetic systems (37), a rapid and inexpensive screening procedure has been proposed using embryonic chick (69). The chick embryotoxicity screening test – CHEST is based upon the caudal morphogenetic system. In this technique agents are administered directly below the caudal region of the embryo, which is at Hamburger Hamilton stage 10 or 11. After a 24 hours incubation, the length of the caudal trunk is measured and used as a quantitative estimate of embryotoxicity. The procedure consists of three steps. The first step (CHEST I) performed on the caudal MGS serves for the identification of general cytotoxic properties. The second step (CHEST II) specifies the dose effect relationships, the stage effect and consequently the receptor mediated effect if present. The third step (CHEST III) enables one to screen for the specific human embryotoxic metabolites. Using CHEST, an embryotoxic affect level can be easily determined for any substance and related to the maximal intended therapeutic or exposure dose. The predictive values of CHEST seems to be as good (or as bad) as that of the current routine procedures on mammalian species (70).

Iyengar (1983) has developed a new *in situ* organ culture technique using the early chick blastoderm (71). She has studied this early chick embryo blastoderm as a convenient organ culture method for proliferation, differentiation as well as for cell interaction studies. A new window technique has been evolved, freshly laid embryonated eggs are incubated for 17 hours. A hole is created with a sharp straight cutting needle at a point one third the length from the broad end of the egg. With the help of an iris scissors an oval window 1.5 × 1 cm in size is cut. The blastoderm at this stage is seen floating

uppermost on the yolk. Thin albumin is pipetted out from the side so that the level of the embryo is lowered well below the level of the window. This prevents the embryo from being damaged while sealing the window and prevents it from drying when incubated. The window is sealed with broad cellotape and eggs are reincubated and the embryo can be harvested at any time interval required.

Beyond the 40 hours stage new tissue vitiate the observations. Iyengar (1982) has utilized the chick embryo blastoderm for observing the effect of copper on various cancer chemotherapeutic agents (72), Iyengar and Lal (1985) have used the early chick embryo as a model for differentiation and proliferation to study the effect of methylene blue as an organised system (73). It shows that the early chick embryo blastoderm model serves as an actively proliferating organ culture. It serves as a convenient organ culture for the study of cell proliferation, cell interaction and differentiation. As this is in ovo experiment, there is the great advantage of observing cellular events without disturbing the environment. This model can serve as a model for the study of teratology and embryology as it has the combined advantage of *in vitro* tissue culture technique as well as those of a well controlled interactive cell system, it may help in studying the teratogenic potential of drugs. This model has been used by Kotwani et al to study the effect of some drugs on neural tube formation. In this model the incubation time is confined to the period from 17 to 40 hour, as at the 17 hour stage, the embryo consists of three cell layers. Neural tube formation, which is complete at 40 hour serves as a marker of interactive processes during incubation. Injections are made into the sub-blastodermal space (that ensures drug delivery in the embryo) at 17 hours (window is created), using a 23G needle and tuberculin syringe. Injections are given in a constant volume of 0.06 ml (as this did not give rise to neural tube defects). It has been shown that the chick embryo blastoderm is very sensitive e.g. normal neutral tube (Fig. 2) development is seen when normal saline is injected at 37°C, hot (50°C) or cold (10° and 30°C) saline produces maldevelopment of neural tube (74). Thalidomide, a known teratogen when injected in 6 µg and 30 µg dose produced dose related effect in neural tube defects

Fig. 2 : Normal neural tube development of chick embryo uptil 40 hours (stage II) x 25.

(75). Asprin was injected in four doses and it produced dose related effect in neural tube formation in chick embryo (Figs. 3 and 4) and the effect of aspirin could be antagonised by prior administration of $PGF_{2\alpha}$ (76). So in this model we can study the effect of drug and can find out the possible mechanism by giving another drug which can block the action of tested drug. Diflunisal which is like aspirin a salicylic acid derivative, also produced neural tube defects but its action was not antagonised either by PGE_1 or $PGF_{2\alpha}$ (77). It has also

Fig. 3 : Anterior end defect in neural tube development (uptil 40 hours) of chick embryo after aspirin tratment x 25.

Fig. 4 : Both endes defect in neural tube development (uptil 40 hours) of chick embryo after aspirin or diflunisal treatment x 25.

shown that if the drug solution which is injected has low pH (2.6–3.1), it would produced more defects in neural tube development (78). So chick embryo blastoderm model can act as a prescreen test for testing teratological potential of various new compounds. This model fulfills all the criteria which a test should have at a lower level of tier system in teratological studies i.e. it is inexpensive, short incubation time, small size, known embryological development, ease of accessibility to the embryo, possibility of experimenting on a large scale for statistically valid results,

does not require sophisticated gadget or specialized trained personnel, whole animals are also not required.

Chick embryo has also been used to study cardiovascular teratogenicity of various compounds. Trichloroethylene and dichloroethylene are industrial solvents and are frequently found as drinking water contaminants and have been shown to produce cardiac teratogenicity in chick model (79). In this method White Loghorn chick eggs are inoculated just above the embryo with 30 µl of a test solution on day 3 of incubation. Chicks are terminated on day 18 of incubation and effect is seen on heart and great vessels. In an another method to study cardiovascular teratogenicity topical method of application of drugs (e.g. terbutaline or ritodrine) is used at stage 24 (4–day) in chick embryo (80). To study the mechanism of action of drug pretreatment with blockers (e.g. butaxamine or metoprolol) 4 hours before application of agoinst can be studied. Eggs are allowed to incubate until stage 41 (day 15) and then effect is observed on heart and great vessels. Similarly, there are many other reports in which many other drugs/ agents like methylxanthines, ephedrine have been used to study their cardiac toxicity in chick embryo and interaction with forskolin has been studied (81–82). Chick embryo has also been used by many other scientists to study the effect of various drug e.g., interaction of verapamil and metoprolol (83) or the effect of Palyam serogroup orbiviruses (84) by utilising the above mentioned model i.e. injecting the drug on day 4.

Ablation of pre-migratory cardiac neural crest has also been used to produce and study extensively a model of abnormal cardiovascular dysmorphology in chick embryos. Gale and Kirby have studied different aspects of the involvement of cranial neural crest in the development of cranial, cervical and cardiac tissues in chick embryos (85). Recently it has been shown in chick embryo that folate deficiency can produce congenital defects of the heart and neural tube (86). Folate deficiency can increase the concentration of homocysteine which is a teratogen *per se* and can produce teratogenic effect.

Pathogenesis of caudal dysgenesis/ sirenomelia has also been studied in chick embryos. Wei and Sulik have described the particular vulnerability of specific caudal structures to ochratoxin A, a fungal toxin in chick embryo (87). Injection was given into the air sac of egg after incubating it for 48 hours and then at different time periods the effect of fungal toxin was studied.

Effect of MR exposure at 1.5 T and to 64–MHz on early embryonic development of the chick was studied by Yip et al (88). MR exposure was given within first 42 hours of incubation for 4 hours. Embryos did not show any significant development defect if sacrificed shortly thereafter but there was a trend toward higher abnormality and mortality rate when embryos were sacrificed on the 6th day of incubation. An interesting interaction of magnetic field (MF) 50 Hz and X-ray or drugs have been studied by Pafkova et al (89). There is no significant alteration of chick embryotoxicity after repeated exposures to 50 Hz MF at 10 m T or 6 micro T or with different vectors. A decrease of X-ray induced teratogenicity was observed when MF preceded X–ray exposure, while MF exposure applied immediately after

X-ray radiation non-significantly potentiated adverse developmental effects of ionizing radiation. Similar results were obtained with MF and insulin or tetracycline.

CONCLUSION

Chick embryo is a useful method for studying the teratogenic potential of new compound. It can be used as part of a battery of *in vitro* tests for teratogens. Information from *in vitro* tests can be usefully used as a component of the risk/ hazard assessment process (90). Hence, instead of being alternative to testing in animals, chick embryo can serve as efficient prescreen to rank chemicals so that only those few with a high hazard potential may be submitted to detailed developmental toxicity testing in pregnant animals.

REFERENCES

1. Kotwani A, Mehta VL, Gupta U, Prabhu S, Bapna JS. Methods for teratogenicity testing - existing and future models. *Ind J Pharmacol* 1995; 27: 204–213.
2. Wilson JG. Current status of teratology - General principles and mechanisms derived from animal studies. In : Wilson JG, Fraser FE, eds. Handbook of teratology Vol.1 New York; Plenum Press, 1977: 47–74.
3. Brown NA, Fabro SE. The *in vitro* approach to teratogenicity testing. In : Snell K ed. Development toxicology Vol.1 New York; Praeger Publications, 1982; 33–57.
4. Goldberg AM. Integration of fundamental knowledge and *in vitro* testing strategies. In : Homburger F, Goldberg AM eds. *In vitro* embryotoxicity and teratogenicity test. *Basel. Karger* 1985; 1–5.
5. Lumley CE. Proposal for international guidelines for reproductive and developmental toxicity testing for pharamaceuticals. *Adverse Drug React Toxicol Rev* 1991; 10: 143–153.
6. New DAT. Whole embryo culture and the study of mammalian embryos during embryogenesis. *Biol Rev* 1978; 53: 81–122.
7. Gebhardt DOE. The use of the chick embryo in applied teratology. In: Wollam DHM,ed. Advances in teratology. London: Academic Press, 1972; 5: 97–111.
8. Birge WJ, Black JA, Westerman AG, Ramey BA. Fish and Amphibian embryos – a model system for evaluating teratogenicity. *Fund Appl Toxicol* 1983; 3: 237–242.
9. Sakamoto MK, Mima S, Kihara T, Matsuo T, Yasuda Y, Tanimura T. Developmental toxicity of caffeine in the larvae of xenopus larvis. *Teratology* 1993; 47: 189–201.
10. Bournias - Vardiabasis N, Teplitz RL. Use of drosophila embryo cell culturs as an *in vitro* teratogen assay. *Teratogenesis Carcinogen Mutagen* 1982; 2: 335–341.
11. Waltan BT. Use of the cricket embryo (Acheta domesticus) as an invertebrate teratology model. *Fund Appl Toxicol* 1983; 3: 233–236.
12. Johnson EM, Gabel BEG. An artificial embryo for detection of abnormal developmental biology. *Fund Appl Toxcol* 1983; 3: 243–249.
13. Whitby KE. Teratological research using *in vtro* system. III embryonic organs in culture. *Environ Health Perspect* 1987; 72: 221–223.
14. Elstein KH, Zucker RM, Shney DL, Hau C, Chernoff N, Rogers JM. Utility of the murine erythroleukemic cell (MELC) in assessing mechanisms of action of DNA active developmental toxicants. Application to 5 fluorouracil. *Teratology* 1993; 48: 75–87.
15. Karnofsky DA. The chick embryo in drug screening. Survery of teratological effects observed in the 4–day chick embryo. In: Wilson JG, Warkay J eds. Teratology: Prinicples and Techniques. Chicago. Univ Chicago Press, 1965 194–261.
16. Freeman BM, Vince MA. Development of the avian embryo London: Chapman and Hall, 1974: 1–156.
17. Patten BM. Early embryology of the chick. 4th ed. New York: Mc Graw-Hill Book Company, 1961; 1–116.

18. Hamburger V, Hamilton HL. A series of normal stages in the development of chick embryo. *J Morphol* 1951; 88: 49–92.

19. Rohlich GA ed. Drinking water and health, Report of the safe drinking water committee. *Nat Acad Sci Washington DC* 1977.

20. Dareste C. "Recherches sur la production artificielle des monstruosites ou Essais de teratogenic experimentale" Paris: C. Reinwald, 1877.

21. Wilson JG. Survey of *in vitro* systems: Their potential use in teratogenicity screening. In : Wilson JG, Fracer FC eds. Handbook of Teatology, Vol. 4, New York: Plenum Press, 1978 135–158.

22. Marliac JP. Injections of chemicals into chicken eggs as a toxicity test. *Fed Proc* 1962; 21: 450.

23. Mc Laughlin J Jr., Marliac JP, Verrett MJ, Nutchler NK, Fitzhug OG. The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. *Toxicol Appl Pharmacol* 1963; 5: 760–771

24. Khera KS, Lyon DA. Chick and duck embryos in evaluation of pesticide toxicity. *Toxicol Appl Pharmacol* 1968, 13: 1–15.

25. Hanan EB. Absorption of vital dyes by the fetal membranes of the chick. *Am J Anat* 1927; 38: 423–450.

26. Van Kaick G, Goerttler K. Technik and probleme teratologischer studien am bebruteten Huhherkeim. *Klin Wochenschr* 1968; 46: 959–960.

27. WHO. Principles for the testing of drugs for teratogenicity. *Tech Rep Ser* 364. Geneva, WHO, 1967.

28. Jelinek R. Use of chick embryo in screening for embryotoxicity. *Teratogenesis Carcinogen Mutagen* 1982; 2: 255–261.

29. Jelinek R, Dostal M. Species specificity in teratology in the light of analyzing the intraspecies differences in mice. *Folia Morphol (Prague)* 1973; 21; 94–96.

30. Gillett JR. Factors that affect drug concentrations in maternal plasma. In: Wilson JG Fraser FC eds. Handbook of teratology, Vol.3, New York, Plenum Press, 1977: 79–115.

31. Puchkov VF, Popou VB, Jelinek R, Dostal M. Comparative assessment of the efficiency of severval testing system in evaluating the embryotoxicity of cyclophosphamide. *Arkh Antat Gistol Embriol* 1981; 80: 104–110.

32. Jelinek R, Peterka M. Morphogenetic systems and in vitro techniques in teratology. Paper presented at the 5th symp on prenatal development, Berlin, May 7–9, 1981.

33. Jelinek R, Benesova O, Peterka M, Soucek K, Zatecka I. Embryotoxicity of maprotiline (Ludiomil, Ciba-Giegy) in a semiclinical experiment. *Act Nerv Super (Prague)* 1978; 20: 300.

34. Walker NE. Distribution of chemical injected into fertile eggs and its effect upon apparent toxicity. *Toxicol Appl Pharmacol* 1967; 10: 290–299.

35. Zageis N, Georgastros JG. Application of chemicals in early chick embryos in ovo: A precaution. *Experientia* 1977; 33: 1255.

36. Verrett MJ, Scott WF. Reynaldo EF, Alterman EK, Thomas CA. Toxicity and tertogenicity of food additive chemicals in the developing chicken embryo. *Toxicol Appl Pharmacol* 1980; 56: 265–273.

37. Jelinek R, Ruchter Z. Morphogeneitc systems and the central phenomena of teratology. In: Persaud TVN ed. Advanes in the study of birth defects vol. II: Teratological testing. Balimore. University Prak Press, 1979: 41–67.

38. Rychter Z, Jelinek R. Foundations of experimental teratology. *Prague: Avicenum* 1978: 159

39. Schowing J. Teratogenic effects of cadmium acetate and sulfate upon development of the chick embryo. *Acta Morphol Hung* 1984; 32: 37–46.

40. Waddington CH. Experiments on the development of chick and duck embryos cultivated *in vitro*. *Phil Trans R Soc* 1932, 221: 179–230.

41. Spratt NT. Development *in vitro* of the early chick blastoderm explanted on yolk and albumen extract slaine agar substrate. *J Exp Zool* 1947; 106: 345.

42. New DAT. A new technique for the cultivation of the chick embryo *in vitro*. *J Embryol Exp Morph* 1955; 3: 320–321.

43. Wolf E, Simon D. L`explantation et la parabiase in vitro de blastodermes incuber d` embryons de paulet. L`organisation de la circulation extra embryonnaire. *CR Hebd Seanc Acad Sci Pans* 1955; 241: 1994–1996.

44. Dunn BE. Techinque for shell-less culture at the 72 hour avian embryo. *Poult Sci* 1974; 55: 1067–1071.

45. Krucera P, Burnand MB. Routine teratogenicity test that uses chick embryos *in vitro*. *Teratogen Carcinogen Mutagen* 1987; 7: 427–447.

46. Kucera P, Burnand MB. Teratogenicity screening in standrdized chick embryo culture: effects of dexamethosone and diphenylhdantoin. *Experientia* 1988; 44: 827–833.

47. Folkman J. Tumor angiogenesis. In: Klein G, Weinhouse S eds. Advances in cancer research. New York. Academic Press, 1974; 331:358.

48. Klagsbrum M, Knighton D, Folkman J. Tumor angiogenesis activity in cells grown in tissue culture. *Cancer Res* 1976; 36: 110–114.

49. Crum R, Szabo S, Folkman J. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* 1985; 230: 1375–1378.

50. Auerbach R, Kobai L, Knighton D, Folkman J. A simple procedure for the long term cultivation of chicken embryos. *Developmental Biol* 1974; 41: 391–394.

51. Dunn BE, Fitzharris TP, Barnett BD. Effects of varying chamber construction and embryo pre-incubation age on survival and growth of chick embryos in shell less culture. *Anta Rec* 1981; 199: 33–43.

52. Jakobson AM, Hahnenberger R, Magnusson A. A simple method for shell less cultivation of chick embryos. *Pharmac Toxicol* 1989; 64: 193–195.

53. Karnofsky DA. The use of the developing chick embryo in Pharmacologic research. *Stanford Med Bull* 1955; 13: 247–59.

54. Kaplan S, Grabowski CA. Analysis of trypan blue induced runplessness in chick embryos. *J Exp Zool* 1967; 165: 325–336.

55. Gebhardt DOE. The teratogenic action of propylene glycol (propanediol-1,2) and propanediol 1,3 in the chick embryo. *Teratology* 1968; 1: 153–161.

56. Goel SC, Jurand A. Effects of hydrocortisone acetate on the development of chicken embryos. *Teratology,* 1976; 13: 139–149.

57. Grubb RB, Montiegel EC. The teratogenic effects of 6-mercaptopurine on chick embryos in ovo. *Teratology* 1975; 11: 179–185.

58. Hall BK. Thallium-induced achondroplasia in chicken embroys and the concept of critical periods during development. *Teratology* 1977; 15: 1–16.

59. Overman DO, Graham MN, Roy WA. Ascorbate inhibition of 6–aminonicotinamide teratogenesis in chicken embryos. *Teratology* 1976; 13: 85–93.

60. Mieniel R. Action protectrice de la Pralldoxime vis-a-vis des effects teratogenes du parnathion sur le sequelette axial de l' embryon de caille. *CR Acad Sci Sec D* 1974; 279: 603–606.

61. Hodasch RJ, Gilbert EF, Fallon JF. Aortic arch anomalies associated with the admilnistration of epinephrine in chick embryos. *Teratology* 1974; 9: 203–209.

62. Landauer W. Cholinomimetic teratogens. Studies with chicken embryos. *Teratology* 1975; 12: 125–145.

63. Schom CB, Abbott UK. Temporal morphological and genetic responses of avian embryos to azodrin. an organophosphate insecticide. *Teratology* 1977; 15: 81–87.

64. Romanoff GA. The Avian Embryo: Structure and Functional Development. New York: Macmillan Co., 1960.

65. Mann RA, Moore KL, Persaud TVN. Limitations in the use of the early chick embryo as a teratological model. *Teratology* 1973; 7:A22–23.

66. Mann RA, Persaud TVN. Embryogenesis of open neural defects in the chick embryo. *Anat Rec* 1978; 190: 468–469.

67. Mann RA, Persaud TVN. Histogenesis of experimental open neural defects in the early chick embryo. *Anat Anz* 1979; 146: 171–187.

68. Fisher M, Schoenwolf GC. The use of early chick embryos in experimnetal embryology and teratology : improvements in standard procedures. *Teratology* 1983; 27: 65–72.

69. Jelinek R. The chick embryotoxicity screening test (CHEST). In Neubert D, Merker HJ, Kwasigroch TE, Eds. Methods in prenatal toxicology. *Thieme: Struttgart* 1977: 381–386.

70. Marhan O, Jelinek R, Rychter Z, Rezabek K. Seventeen drugs compared in embryotoxicity tests using pregnant animals and a morphogeneic system. In: Benesova O, Rychter Z, Jelinek R eds. Evaluation of embryotoxicity, mutagenicity and carcinogenicity risk in new drugs. *Praha : Univerzita Karlova,* 1979, 219–227.

71. Iyengar B. A new *in situ* organ culture technique using the early chick blastoderm. *Chemotherapy* 1983; 29: 68–70.

72. Iyengar B. Copper levels and the adriamycin inhibition of proliferating cells. *Chemother Pharmacol* 1983; 10: 227.

73. Iyengar B, Lal SK. Methylene blue and organised differentiation in the chick embryo. *Acta Anat* 1985; 123: 220–223.

74. Kotwani A, Mehta VL, Iyengar B. Effect of variations in temperature of injected saline on neural tube formation in chick embryo. *Ind J Exp Biol* 1992; 30: 295–298.

75. Kotwani A. Effect of some newer analgesic and anti-inflammatory drugs on proliferation differentiation in chick blastoderm organ culture (Thesis). New Delhi: Univ of Delhi, 1992.

76. Kotwani A, Mehta VL, Iyengar B. Mechanisms of aspirin induced neural tube defect in chick embryo. *Ind J Med Res* 1994; 99: 289–294.

77. Kotwani A, Mehta VL, Iyengar B. Diflunisal affects chick embryo neural tube development – an action not mediated by prostaglandins. *Ind J Pharm* 1997; 29: 117–124.

78. Kotwani A, Mehta VL, Iyengar B. Aspirin by virtue of its acidic property may act as teratogen in early chick embryo. *Ind J Physiol Pharmac* 1995; 39: 131–134.

79. Goldberg, SJ, Dawson BV, Johnson PD, Hoyme HE, Ulreich JB. Cardiac teratogenicity of dichloroethylene in a chick model. *Ped Res* 1992; 32: 23–26.

80. Lenselink DR, Kuhlmann RS, Lawrence JM, Kolesari GL. Cardiovascular teratogenicity of terubtaline and ritodrine in the chick emrbyo. *Am J Obstet Gynecol* 1994; 171: 501–506.

81. Nishikawa I, Kasajima T, Kanai T. Potentiating effects of forskolin on the cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol Lett* 1991; 56: 145–150.

82. Nishikawa T, Kasajima T, Kanai T. Interactions between verapamil and metoprolol in the developing chick embryo heart. *J Appl Toxicol* 1991; 11: 111–114.

84. Whistler T, Swanepoel R. Teratogenicity of the Palyam serogroup orbiviruses in the embryonated chicken model. *Epidemiol Infect* 1991; 106: 179–188.

85. Gale TF, Kirby ML. Absence of correlation between transient cranial hemorrhages and congenital malformations following neural crest ablation in chicks. *Teratology* 1996; 53: 318–325.

86. Rosenquist TH, Ratashak SA, Sehub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. *Proc Natl Acad Sci* 1996; 93: 15227–15232.

87. Wei X, Sulk KK. Pathogenesis of Caudal dysgenesis/Sirenomelia induced by Ochratoxin A in chick embryos. *Teratology* 1996; 53: 378–391.

88. Yip YP, Capriotti C, Talagala SL, Yip JW. Effects of MR expousre at 1.5T on early embryonic development of the chick. *J Magn Reson Imagine* 1994; 4: 742–748.

89. Pafkova H, Jerabek J, Tejnorova I, Bednar V. Developmental effects of magnetic field (50 Hz) in combination with ionizing radiation and chemical teratogens. *Toxicol Lett* 1996; 88: 313–316.

90. Flint OP. *In vitro* tests for teratogens : desirable endpoints. test batteries and current status of the micromass teratogen test. *Reprod Toxicol* 1993; 7: 103–111.